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A Research Note

Effect of Dose on Gamma Irradiation Induced Formation of Free Radicals in Freeze-Dried Natural Actomyosin

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ABSTRACT

Freeze-dried preparations of actomyosin from chicken breast muscle were irradiated in liquid nitrogen with gamma radiation from a cesium-137 source at 5, 10, 15, 20, 30, 40, and 50 kGy. The ESR spectra at −130°C were recorded with an EPR spectrometer. Signal intensities increased linearly with radiation. Analysis of data indicated a ten-fold rise in dose resulted in an almost seven-fold increase in number of free radicals generated. Determining the extent of such interaction on a major structural protein can help assess type and extent of reactions which might occur on irradiation of animal products.

INTRODUCTION

IONIZING RADIATION during irradiation of food generates free radicals. Myofibrillar proteins, large polymeric constituents in muscle tissue, are particularly sensitive to ionizing radiation. Electron spin resonance spectroscopy (ESR) has been used to study free radical formation in protein-rich foods (Taub et al., 1978; Uchiyama and Uchiyama, 1979), in dry and moist collagen (Shieh and Wierbicki, 1985) and in myosin and actomyosin (Taub et al., 1979a; 1979b; 1979c; Taub, 1981).

Quantitative data on free radical formation in irradiated proteins are limited. The ESR signal for irradiated myosin and actin reached a constant value at doses above 200kGy (Taub et al., 1979c). The concentration of free radicals in irradiated gelatin increased linearly or exponentially within the tested dose range of 10-100 kGy (Bachman et al., 1974).

The objective of our study was to examine free radical formation caused by gamma irradiation, in a freeze-dried preparation of natural actomyosin from broiler chicken breast muscle. This information is needed to assess possible effects of radiation treatment of meats and poultry. A major value of this investigation will be to help predict type and extent of reactions which may occur in irradiated muscle foods.

MATERIALS & METHODS

Materials

Freshly killed broiler chickens were purchased from a commercial processor and stored at 4°C 24 hr. Breasts were removed, and muscle tissues separated from skin and adipose tissue. The breast muscle was ground through a 3 mm plate and homogenized with 6 volumes of Hasselbach-Schneider solution (ionic strength = 0.76) at pH 6.4 (0.6M KCl, 0.1M KH_2PO_4, 0.1M K_2HPO_4, 0.01M $Na_4P_2O_7$, 10mM $Na_4P_2O_7$, 10 mM $MgCl_2$) in a MPW 302 homogenizer at medium speed 1 min. The suspension was centrifuged 15 min, and the resultant precipitate discarded. The supernatant was diluted with double-distilled water to ionic strength 0.3, to precipitate actomyosin (Perry, 1955). After 24 hr at 4°C the sediment was redissolved with an appropriate volume of Hasselbach-Schneider solution. This solution was centrifuged and dissolved actomyosin was reprecipitated over 2 hrs by readjusting ionic strength to 0.3. This step was repeated twice. After final centrifugation, the actomyosin gel was washed 2x with double-distilled water to remove chloride and avoid probable radiolytic formation of Cl^- free radicals (Taub et al., 1979c). The actomyosin gel was freeze-dried and stored at −20°C.

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Irradiation

About 15 mg of freeze-dried actomyosin were placed into Amersil glass tubes, immersed in liquid nitrogen and irradiated with ^{137}Cs at 0.10 kGy/min. Three samples were irradiated at each dosage: 5, 10, 15, 20, 30, 40, and 50 kGy. Immediately after irradiation samples were removed and ESR spectra obtained.

ESR measurement

ESR spectra were recorded with a Varian Associates EPR spectrometer model E 109B equipped with frequency counter and IBM System 9000 for ESR data acquisition. General EPR spectral parameters were: modulation amplitude, 1.0 gauss; time constant, 0.128 sec; scan time, 4 min; microwave power, 2 mW. All spectra were recorded and conducted at 143°K. Data were integrated and normalized; DC and linear baseline correlations were used. To determine the concentration of spins/mg of the preparation, spectra were compared with that of a bar-shaped sample of $Al_2O_3:Cr^{3+}$ (ruby) of *Standard Reference Material 2601* (National Bureau of Standards, 1978). The g-value was calculated from the formula $g = h\nu/\beta H$ relative to the standard, α, α-diphenyl-β-picryl hydrazyl (DPPH), and the frequency (ν) measured with a HP frequency counter. Alternatively, the magnetic field strength was calibrated relative to potassium peroxyamine disulfonate (A_n = 13.0 gauss and g = 2.0056).

RESULTS & DISCUSSION

THE ESR SPECTRUM of irradiated actomyosin (Fig. 1) showed an anisotropic signal with a subsignal and a major signal with line width 21 gauss and g-value 2.004. Increasing dosages of radiation caused an increased number of free radicals (Table 1). The effect of dose on number of spins per mg of actomyosin (the concentration of free radicals is directly proportional to radical concentration) could be fitted to the expression $y = ax + b$. The slope of the straight line was 2.5518×10^{13}, y intercept = 13.023×10^{13}, and r = 0.99. The least squares line did not go through the origin. This could be readily explained considering the distribution of reactive sites in the material. Based on the slope of the line describing the relationship between dose and number of spins, a G-value of 0.41 was obtained after correction for electron volts and doses. The G-value refers to number of radicals (spins) produced per 100 ev of energy absorbed in the sample. Additionally, the total absorbed dose equals the transient dose plus the exposure dose. The transient dose was usually determined by extrapolation of the curve to zero exposure time. A number of reactive sites may lie on or near the surface of the material and upon irradiation "instantly" form radicals.

The data do not fit a first or second order rate equation. This can be explained by considering the reactivity between free radicals and proteins. If we assume the ionizing radiation is sufficiently energetic to react with any reactive site and form a radical, then the limiting step is the total number of reactive sites present. When all possible sites have reacted, the number

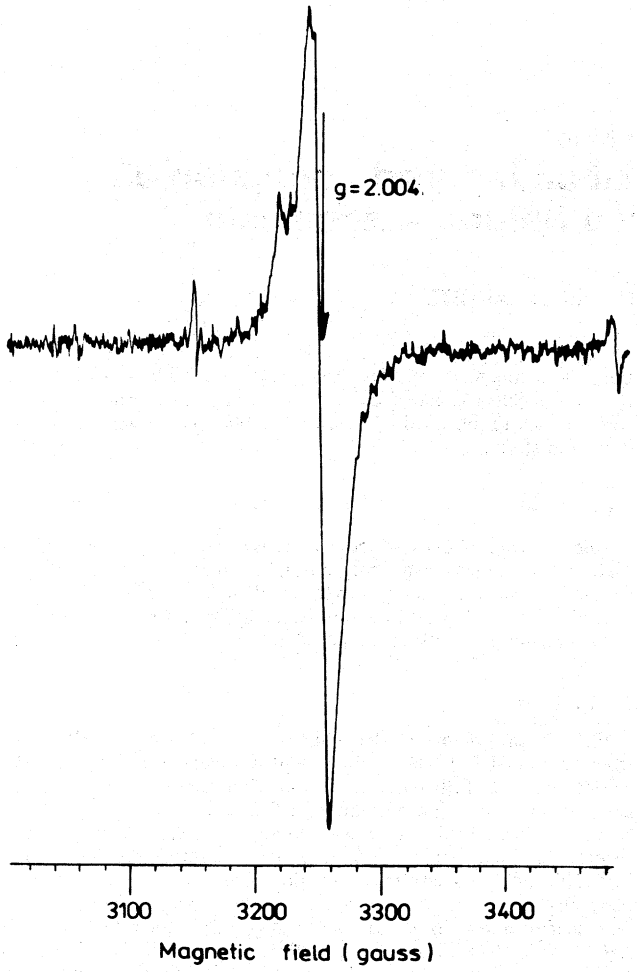

Fig. 1—ESR spectrum of actomyosin irradiated at 10 kGy.

Table 1—Effect of dose on the relative ESR signal intensities and spin concentration in freeze-dried preparations of actomyosin

Dose kGy	Relative signal intensity[a]	Number of spins per mg/x 10^{-13}
5	2.4921 ± 0.0843[b]	20.6953 ± 0.8533[b]
10	4.1167 ±0.2436	36.4960 ± 2.1745
15	5.7394 ± 0.2844	50.8830 ± 2.5379
20	8.4457 ± 0.8088	74.8745 ± 7.2189
30	10.1503 ± 0.3760	89.9872 ± 3.5580
40	12.5695 ± 0.9631	114.4343 ± 8.5944
50	15.5236 ± 3.0299	137.6223 ± 27.0413

[a] Normalized, corrected for the sample weight.
[b] Mean values of 3 samples ± standard error of a mean.

of radicals will approach a maximum. This usually occurs at higher dose levels and has been observed as an asymptotic curve (Simic, 1978; Taub et al., 1978; Prydz and Henriksen, 1961; Uchiyama, 1979).

The spectrometric g-value calculated for the major peak in Fig. 1 is 2.004, agreeing with the value obtained by Uchiyama and Uchiyama (1979) in irradiation studies with protein-rich fish cakes. The ESR signal is attributed to carbon-centered radicals that presumably may derive from the carbon backbone of actomyosin. This hypothesis would correspond with findings by others (Van Paemel et al., 1975; Taub et al., 1979a; 1979b; 1979c; Taub, 1981). The ESR spectra in our study differ from spectra of amino acids with sulfur, groups which, produce ESR spectra with larger g-values (Bielski and Gebicki, 1967).

Results from our investigation can be extrapolated to other muscle proteins to understand reactivity of free radicals and structural proteins. In our study reactivity and free radical decay were reduced, since the preparations were kept in liquid nitrogen during irradiation. The interaction of free radicals during ESR measurements would also be eliminated, because at −130°C rotational motion of residual water molecules is negligible (Taub et al., 1978).Electron spin resonance evidence shows that H· starts to decay around −253°C and OH· −163°C, (Siegel et al., 1960). Under those experimental conditions, and radiation levels formation of free radicals was a linear function of dose. These data may be useful in predicting extent and type of reactions which may occur when similar muscle protein is irradiated. Our method however, can only be applied to study dried samples.

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